RESEARCH ARTICLE

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Stromal Tumor-Infiltrating Lymphocytes Associated with Immunohistopathology and Molecular Subtypes of Breast Cancer in Vietnam

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Abstract

Objective: Breast cancer is a heterogeneous disease with varied symptoms and pathogenesis, as well as variable prognosis and therapeutic outcomes. Stromal tumor-infiltrating lymphocytes, one of the tumor microenvironment factors, has been recognized as an important immunological biomarker that reflected the antitumor immune response in breast cancer. **Methods:** We analyzed 207 invasive breast cancer patients who had lumpectomy or mastectomy and have not received any pre-operative treatment. Clinicopathological characteristics, immunohistochemistry characteristics, molecular subtypes classification and stromal TILs evaluation were investigated. **Result:** Stromal TILs correlated with well-established prognostic markers. Tumor grade showed significantly higher sTILs percentages in high-grade tumors than in low-grade tumors ($p<0.001$). There was a statistically significant association between intermediate and high levels of sTILs and a high Ki-67 index ($p< 0.001$). ER/PR negative was significantly related to high sTILs. Mean sTILs score was significantly higher in TNBC (40.1±31.6%) compared to others, statistically significant ($p<0.001$). In HER2-negative breast cancer, sTILs were significantly associated with histologic grade, ER status, PR status, and Ki67 index. **Conclusion:** sTILs played an important role, associated with unfavorable factors in breast cancer. Our findings support the use of stromal sTILs to identify a more aggressive phenotype of tumors.

Keywords: breast cancer- tumor microenvironment factors- stromal tumor-infiltrating lymphocytes- molecular subtypes

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Introduction

Breast cancer is a significant global public health issue. It accounts for 25% of all cancers -the most frequently identified malignancy and common cause of cancer death in women (Sung et al., 2021). Breast cancer is a heterogeneous disease with varied symptoms and pathogenesis, as well as variable prognosis and therapeutic outcomes. The pioneering works on molecular classification of Perou suggested the molecular categorization of breast cancer based on gene expression patterns in the year 2000 (Perou et al., 2000), subsequently, this molecular classification has also been confirmed by several other researchs (Rouzier et al., 2005; Peppercorn et al., 2008; Anderson et al., 2014). In 2013, IHC-based molecular classification was recommended in the St Gallen guidelines for clinical decision making and pointed out that four molecular subtypes of breast cancer can be identified: Luminal A and Luminal B (hormone receptor positive), and HER2 (HER2 overexpression), and Triple negative (estrogen receptor negative (ER-), progesterone receptor negative (PR-), and HER2 negative (HER2)) (Goldhirsch et al., 2013).

The tumor microenvironment (TME) is the ecosystem that surrounds a tumor comprising a network of tumor cells, blood vessels, lymphatics, myofibroblasts, fibroblasts, neuroendocrine cells, adipose cells, immune-inflammatory cells including myeloid-derived suppressor cells, tumor-associated macrophages, neutrophils, tumor-infiltrating lymphocytes (TILs), and T-cells, as well as the extracellular matrix (Wang et al., 2017; Osipov et al., 2019; Baghban et al., 2020). The TME plays a very important role in cancer tumor progression, evolution, invasion, and metastasis, also tissue microenvironment plays a key role in controlling cell migration, proliferation, polarization, and differentiation (Fidler, 2003; Mott and Werb, 2004). Further, it affects the therapeutic response and resistance of the tumor (Wang et al., 2017; Baghban et al., 2020). A better understanding of TME in cancer could help identify specific pathways and signals in tumor

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differentiation, proliferation, and invasion. Thereby, this contributes to the management of treatment in the future (De Wever and Mareel, 2003). In recent years, the TME has been investigated as a therapeutic target in cancer treatment (Wang et al., 2017; Bejarano et al., 2021).

Development and progression of malignant tumors are characterized by an interaction with the cells in the tumor microenvironment including infiltrating immune cells (Stanton et al., 2016). The immune cell infiltration in breast cancer tissue differed greatly among different breast cancer subtypes and patients (Kurozumi et al., 2019). Lymphocyte-predominant breast cancer (LPBC) is defined as a presentation wherein at least 50% of the tumor tissue is invaded by tumor-infiltrating lymphocytes (TILs), TILs expression could serve as a strong prognostic marker for colorectal and breast cancer, thereafter, many retrospective studies reported that TIL expression in breast cancer could predict the efficacy of drug therapy and prognosis (Issa-Nummer et al., 2013; Denkert et al., 2015). The presence of tumor-infiltrating lymphocytes (TILs), within the tumor and/or in peritumoral sites, has been recognized as an important immunological biomarker that reflected the antitumor immune response in BC, as well as in other malignancies, including colorectal, ovarian and endometrial carcinomas (Denkert et al., 2018; Bai et al., 2021; Hudry et al., 2022; Kono-Sato et al., 2022). Recent studies have shown the prognostic and predictive importance of TILs in breast cancer (Salgado et al., 2015; Denkert et al., 2018; Dieci et al., 2018; Basu et al., 2019).

TILs are classified into stromal (sTILs) and intratumoral (iTILs) TILs, both located within the tumor tissue; however, while sTILs are composed of lymphocytes dispersed in the tumor stroma, up to and including the invasive front of the tumor, iTILs are made up of lymphocytes properly located within the tumor nests and therefore in direct contact with neoplastic cells (Salgado et al., 2015; Denkert et al., 2018). sTILs represent a more reproducible parameter because they are more frequently encountered and therefore easier to detect in hematoxylin and eosin (H&E)-stained sections with no use of immunohistochemical methods (Denkert et al., 2010; Salgado et al., 2015; Denkert et al., 2018), to date, there is no standardized method for evaluating TILs in daily histopathological practice. In 2010 a method of evaluating TILs on needle biopsies samples was proposed by Denkert et al. (Denkert et al., 2010) and, since then, it has been widely accepted. In 2014 an International TILs Working Group introduced a set of recommendations for a standardized evaluation of TILs in BC (Salgado et al., 2015). Recent studies have revealed tumor infiltrating lymphocytes (TILs) to be a promising predictive biomarker for therapy response (Denkert et al., 2010; Castaneda et al., 2016). Tumor infiltrating lymphocytes are the infiltration cytotoxic lymphocytes into the tumor as a host immune response (Anichini et al., 1987; Naukkarinen and Syrjanen, 1990). Several studies have attempted to determine the prognostic value of TILs in breast cancer. In detail, increased TIL levels have been linked to better response to neoadjuvant chemotherapy and with improved survival for patients with triple negative tumors (TNBC) and HER2-positive BC (Denkert et al., 2015). On the other hand, the prognostic and predictive value of TILs in luminal breast cancer remains poorly understood. Based on the above mentioned acquisitions, the aim of the present study was to evaluate the role of sTILs in molecular subtypes of breast cancer. Associations between sTILs distributions and clinicopathological factors such as hormone receptors status and Ki-67 proliferative index have also been investigated.

Materials and Methods

We performed a cross-sectional descriptive study with 207 patients who were diagnosed with invasive breast cancer (IBC) at Ho Chi Minh City Oncology Hospital in the period from January 2022 to October 2022. The diagnostic criteria of IBC were based on the 2019 World Health Organization (WHO) classification of tumors of the breast (Board, 2019). The patients had lumpectomy or mastectomy and had not received any pre-operative treatment.

Immunohistopathological Characteristics

For the selected cases, the data regarding baseline clinical characteristics and pathological findings were collected. Pathological features including tumor grade, mitosis, lymphovascular invasion, perineural invasion, DCIS, and lymph node metastasis were evaluated on H&E. Tumor grade was assessed according to the Nottingham modification of the Bloom–Richardson system (Board, 2019).

Immunohistochemical tests were performed on the Ventana Benchmark XT automatic staining machine with antibodies ER (DAKO), PR (DAKO), HER2 (DAKO), Ki-67 (MIB1- DAKO).

The ER and PR IHC slides were assessed by the Allred scoring system as per the 2010 American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines (Hammond et al., 2010). The assessment of HER2 IHC slides was done using the ASCO/CAP 2018 guidelines (Wolff et al., 2018). Cases with equivocal HER2 staining on IHC were sent for further examination by FISH and their results were documented. Assessment of the Ki67 proliferation index was done as per the guidelines of the International Ki67 in Breast Cancer Working Group (Nielsen et al., 2021). Cut-off and expression of these antibodies were determined in previous recommendation of St. Gallen 2013 (Goldhirsch et al., 2013).

Molecular Subtypes Classification

We classified the cases into four molecular subtypes based on the current established immunohistochemical surrogate definitions: Luminal A and Luminal B (hormone receptor positive), and HER2 (HER2 overexpression), and Triple negative (estrogen receptor negative (ER-), progesterone receptor negative (PR-), and HER2 negative (HER2) (Goldhirsch et al., 2013; Tang and Tse, 2016).

There is currently no standardized cut-off value established for the Ki67 proliferation index. Although a cut-off of 14% was endorsed in the St. Gallen expert consensus Panel recommendation guidelines in 2011,

the majority of the panel in the St. Gallen 2013 meeting voted a threshold of 20% as indicative of high Ki67 status (Goldhirsch et al., 2013). We have taken a cut-off of 20% as indicative of high Ki67 in the present study.

Stromal TILs evaluation

Although the methods to quantify TIL expression and cut-off TILs values in breast cancer tissues varied among studies and have not been clearly standardized, the International TILs Working Group published the first guidelines for a TIL evaluation in 2014 (Salgado et al., 2015). Accordingly, mononuclear immune cells located between tumor sheets, i.e., within the tumor stroma, are defined as stromal TILs (sTILs). The evaluation of the sTILs was performed by a percentage count of the stromal areas occupied by the lymphocyte and plasma cellular infiltrate, instead excluding the areas occupied by tumor cells. This evaluation considered only the mononuclear infiltration within the borders of the invasive tumors. Large areas of central necrosis or fibrosis are not included in the evaluation. The percentage will be rounded up to the nearest 5%–10%.

The International Working Group (Salgado et al., 2015) recommended that sTILs expression should be gradedbased on their relative abundance within the tumor stromal and devided to:

- Low (sTILs ≤ 10%).
- Intermediate (10% < sTILs <50%).
- High (sTILs ≥ 50%).

The extent of TILs in IBC is gaining importance as a prognostic marker, for quantifying TILs, it is recommended to follow the scoring recommendations (steps) according to WHO classification of breast tumors 2019 (Board, 2019).

Statistical analysis

We presented the data as frequency counts with percentages for categorical variables. Pearson's Chi-square test or Fisher's exact test was used to compare the difference between groups. R program for Windows version 4.1.3 was used to perform all statistical analyses. Statistical significance was set at $p < 0.05$ (2-tailed).

Results

A total of 207 breast cancer cases were diagnosed at The Oncology Ho Chi Minh City in the period from 1/2022 to 10/2022. Among these, the patient's ages at diagnosis ranged from 31 to 80 years old with mean age 54.1 years ±11.4 years and the median age of 48 years.

Immunohistopathological Characteristics

Regarding the histological type, invasive ductal breast carcinoma, no special type was the most common histologic type, accounting for 84.54% of cases. The remaining 15.46% consists of cribriform carcinoma, invasive lobular carcinoma, oncocytic carcinoma, solid papillary carcinoma with invasion, mucinous carcinoma, metaplastic carcinoma, secretory carcinoma, micropapillary carcinoma, adenoid cystic carcinoma and apocrine carcinoma. About histological grade, most tumors were grade II, accounting for 40.58% of cases, followed by grade III (38.16%) and grade I (21.26%) respectively (Table 1).

In our study, ductal carcinoma in situ was seen in 61.4% of cases in our study (127 cases). Out of 207 cases,

Table 1. Clinicopathological Characteristics and Molecular Subtypes

Immunohistopathological Characteristics	Number of patients n (%)
Age (y)	
30-39	17 (8.21)
40-49	60 (29.0)
50-59	67 (32.4)
60-69	47 (22.7)
≥70	16 (7.73)
Histological type	
Invasive Ductal, NOS	175 (84.54)
Other	32 (15.46)
Histological grade	
Grade 1	44 (21.26)
Grade 2	84 (40.58)
Grade 3	79 (38.16)
Lymph node status	
Negative	130 (62.80)
1-3 positive nodes	49 (23.67)
>3 positive nodes	28 (13.53)
Stromal TILs	
Low	108 (52.17)
Intermediate	63 (30.44)
High	36 (17.39)
LVI	
Positive	26 (12.56)
Negative	181 (87.44)
PNI	
Positive	48 (23.18)
Negative	159 (76.82)
ER	
Positive	149 (71.98)
Negative	58 (28.02)
PR	
Positive	111 (53.62)
Negative	96 (46.38)
HER2	
Positive	60 (28.99)
Negative	147 (71.01)
Ki67	
Low (<20%)	75 (36.23)
High (≥20%)	132 (63.77)
Molecular Subtypes	
Luminal A	53 (25.6)
Luminal B	96 (46.4)
HER2	26 (12.6)
TNBC	32 (15.4)

Table 2. Stromal TILs Evaluation Correlated with Other Features

	Total n (%)	Low n (%)	Intermediate n (%)	High n (%)	p.overall
[ALL]	207 (100)	107 (51.69)	64 (30.92)	36 (17.39)	
Age					0.19
30-39	17 (8.21)	9 (8.41)	6 (9.38)	2 (5.56)	
40-49	60 (29.0)	27 (25.2)	18 (28.1)	15 (41.7)	
50-59	67 (32.4)	35 (32.7)	18 (28.1)	14 (38.9)	
60-69	47 (22.7)	29 (27.1)	14 (21.9)	4 (11.1)	
≥70	16 (7.73)	7 (6.54)	8 (12.5)	1 (2.78)	
Histology grade					<0.001
Grade 1	44 (21.3)	32 (29.9)	12 (18.8)	0 (0.00)	
Grade 2	84 (40.6)	47 (43.9)	26 (40.6)	11 (30.6)	
Grade 3	79 (38.2)	28 (26.2)	26 (40.6)	25 (69.4)	
LVI					0.097
Negative	181 (87.4)	97 (90.7)	51 (79.7)	33 (91.7)	
Positive	26 (12.6)	10 (9.35)	13 (20.3)	3 (8.33)	
PNI					0.064
Negative	159 (76.8)	78 (72.9)	48 (75.0)	33 (91.7)	
Positive	48 (23.2)	29 (27.1)	16 (25.0)	3 (8.33)	
DCIS					0.178
Negative	80 (38.6)	42 (39.3)	20 (31.2)	18 (50.0)	
Positive	127 (61.4)	65 (60.7)	44 (68.8)	18 (50.0)	
Lymph nodes status					0.503
Negative	130 (62.8)	69 (64.5)	36 (56.2)	25 (69.4)	
1-3 positive nodes	49 (23.7)	26 (24.3)	17 (26.6)	6 (16.7)	
>3 positive nodes	28 (13.5)	12 (11.2)	11 (17.2)	5 (13.9)	
ER					<0.001
Negative	58 (28.0)	18 (16.8)	21 (32.8)	19 (52.8)	
Weak positive	6 (2.90)	3 (2.80)	1 (1.56)	2 (5.56)	
Positive	143 (69.1)	86 (80.4)	42 (65.6)	15 (41.7)	
PR					0.01
Negative	96 (46.4)	38 (35.5)	34 (53.1)	24 (66.7)	
Weak positive	16 (7.73)	12 (11.2)	3 (4.69)	1 (2.78)	
Positive	95 (45.9)	57 (53.3)	27 (42.2)	11 (30.6)	
HER2					0.09
Negative	147 (71.0)	83 (77.6)	40 (62.5)	24 (66.7)	
Positive	60 (29.0)	24 (22.4)	24 (37.5)	12 (33.3)	
Ki67					<0.001
Low	75 (36.2)	55 (51.4)	15 (23.4)	5 (13.9)	
High	132 (63.8)	52 (48.6)	49 (76.6)	31 (86.1)	
Subtypes					<0.001
Luminal A	53 (25.6)	42 (39.3)	10 (15.6)	1 (2.78)	
Luminal B	96 (46.4)	47 (43.9)	33 (51.6)	16 (44.4)	
HER2	26 (12.6)	9 (8.41)	11 (17.2)	6 (16.7)	
TNBC	32 (15.4)	9 (8.41)	10 (15.6)	13 (36.1)	

lymphovascular invasion was (LVI) seen in 12.56% (26 cases) cases and perineural invasion (PNI) was seen in 23.18% of cases (48 cases). Lymph node metastasis was seen in 77 cases (37.2%). In the present study, ER and PR positivity was noted in 71.98% and 53.62% of the study population respectively. HER2 was positive in 60 cases (28.99%). The Ki67 index high was 63.77% (159 cases) (Table 1).

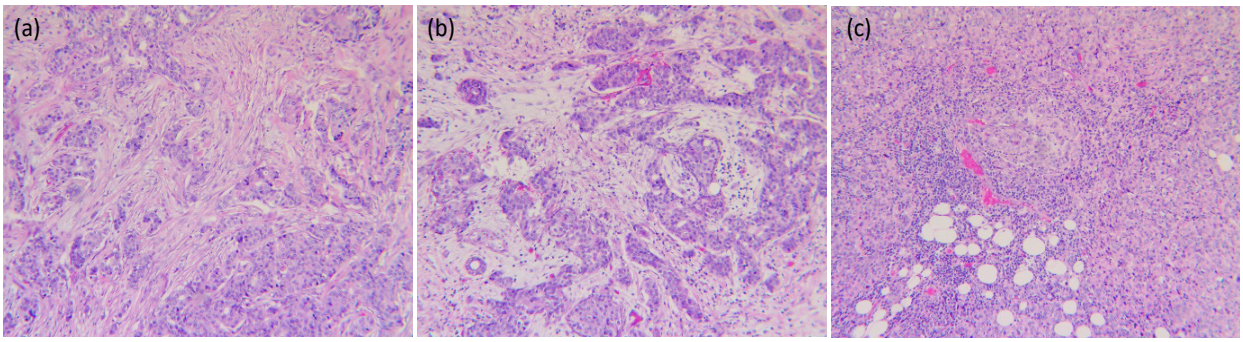

Figure 1. Representative Images of Stromal Tumor-Infiltrating Lymphocytes (sTILs). Low sTILs in (a) (×100), Intermediate sTILs in (b) (×1 00) and High sTILs in (c) (×100).

Table 3. Stromal TILs Evaluation in HER2 Negative Patients

	Total n (%)	Low n (%)	Intermediate n (%)	High n (%)	p.overall
[ALL]	147 (100)	83 (56.46)	40 (27.21)	24 (16.33)	
Histology grade					<0.001
Grade 1	39 (26.5)	30 (36.1)	9 (22.5)	0 (0.00)	
Grade 2	64 (43.5)	37 (44.6)	18 (45.0)	9 (37.5)	
Grade 3	44 (29.9)	16 (19.3)	13 (32.5)	15 (62.5)	
ER					<0.001
Negative	32 (21.8)	9 (10.8)	10 (25.0)	13 (54.2)	
Weak positive	3 (2.04)	2 (2.41)	0 (0.00)	1 (4.17)	
Positive	112 (76.2)	72 (86.7)	30 (75.0)	10 (41.7)	
PR					0.008
Negative	55 (37.4)	22 (26.5)	17 (42.5)	16 (66.7)	
Weak positive	11 (7.48)	8 (9.64)	2 (5.00)	1 (4.17)	
Positive	81 (55.1)	53 (63.9)	21 (52.5)	7 (29.2)	
Ki67					<0.001
Low	66 (44.9)	51 (61.4)	11 (27.5)	4 (16.7)	
High	81 (55.1)	32 (38.6)	29 (72.5)	20 (83.3)	

Molecular Subtypes

Based on the expression of immunohistochemical markers, breast cancer cases were classified into four molecular subtypes. The distribution of these subtypes is presented in Table 1. Luminal B is the most common subtype (46.4%), followed by Luminal A (25.6%), Triple negative (15.4%), and then HER2 (12.6%).

Stromal TILs evaluation

After evaluating the percentage of sTILs, low, intermediate, and high TILs were found in 107 (52.17%), 64 (30.44%) and 36 (17.39%) patients, respectively.

sTILs and Clinicopathological Features Hormone Receptors and Proliferative Index (Ki-67) (Table 2)

Stromal TILs correlated with well-established prognostic markers. Tumor grade showed significantly higher sTILs percentages in high-grade tumors than in low-grade tumors ($p<0.001$). There was a statistically significant association between intermediate and high levels of sTILs and a high Ki-67 index was observed ($p< 0.001$). Moreover, ER/PR negative was significantly related to high TILs ($p< 0.001$ and $p= 0.01$ respectively).

No statistically significant associations have been observed between sTILs status with age, LVI, PNI, DCIS, Lymph nodes status, and HER2 overexpression status.

Stromal TILs distribution across molecular subtypes (Table 2)

Mean sTILs score was significantly higher in TNBC (40.1±31.6%) compared to others, statistically significant ($p<0.001$) (Table 2). We then compared the clinicopathological data according to HER2 overexpression status and analyzed the association between TILs and clinicopathological features of breast cancer based on HER2 status. In HER2-negative breast cancer, TILs were significantly associated with histologic grade, ER status, PR status, and Ki67 index ($p <0.001$, $p <0.001$, $p= 0.008$, $p <0.001$ respectively) (Table 3.), whereas no significant correlation was found in HER2-positive breast cancer.

Discussion

Breast cancer is the leading cause of death in women These prognostic factors are essential for the management and care of breast cancer. Furthermore, molecular classification of breast cancer is now an important tool in patient care guidance. Additonal, sTILs was now considered to be an important predict factor, especially in triple negative molecular subtype. However, the role of sTILs in another subtype was not clear.

Infiltrating tumor lymphocytes are being evaluated in clinical trials as a surrogate marker for treatment response in breast cancer, as summarized recently by TILs working group and specifically in TNBC (Salgado et al., 2015). TILs scores serve as a potential prognostic marker, not only as a surrogate marker to descalate toxic and expensive chemotherapy, but also due to its cost-effectiveness as a diagnostic tool, especially in low-resource countries.

Two comprehensive meta-analyses for stromal TILs association with patient outcomes for large cohorts of breast cancer patients have been studied earlier (Loi et al., 2014; Denkert et al., 2018). Denkert et al., (2018), where pooled data from 6 clinical trials with 3,771 patients where sTILs scores are binned as 60% and above as the cut-off for high sTILs subgroup. In another meta-analysis by Loi et al., sTILs scores for 2148 TNBC patients were analyzed with high sTILs cut-off as 30% and above (Loi et al., 2014). Despite different cut-off for high sTILs at 40%, subtype wise comparison of sTILs scores from our cohort co-related well with reported studies (Salgado et al., 2015; Denkert et al., 2018), where TNBC subtype presented with higher mean sTILs scores compared to that of ER+ and HER2+ subtypes.

Immunohistopathological Characteristics

The mean age at presentation was 54.1 years, which is like other Asia studies but is about a decade lower than that reported in the Western population (Howlader et al., 2014; San et al., 2017, Purushotham et al., 2021). We also found a low proportion of patients presenting at a younger age (40 years) (8.2%) and no patient was under 30 year-olds. Another study, however, observed that 13.2% of their study population comprised of young breast cancer patients (< 40 years) (Nguyen et al., 2021). The majority of our patients were older than 50 years (60.38%) which is similar to the data published in the Vietnam literature (Nguyen et al., 2021).

In the present study, ER and PR positivity was noted in 71.98% and 53.62% of the study population respectively. Most Asia studies report the prevalence of ER/PR positive tumors to be in the range of 50 to 60% (Yamashita et al., 2011; Lin et al., 2014). HER2 overexpression status was seen in 28.99%, higher than other studies (Howlader et al., 2014; Zhu et al., 2014).

Molecular subtypes

The present study assessed the prevalence of molecular subtypes of invasive breast carcinoma in population of Viet Nam. Luminal B-like was the most common molecular subtype (46.4%). A few other studies have also reported a predominance of luminal B subtype in Asia (Yanagawa et al., 2012; San et al., 2017; Kumar et al., 2022). However, our results contrasted those of Nguyen et al., (2021) who reported luminal A as the most common subtype, this study used another criterion.

Stromal TILs evaluation

Stromal TILs scores in the cohort were uniformly distributed irrespective of the clinic-pathological parameters of the tumors, except for grade. Higher sTILs were seen in grade 3 tumors. A similar association was seen by Loi et al., (2014) where a higher sTILs score was significantly associated with high-grade tumors. Within BC, higher sTILs scores co-related with better disease-free outcomes, as reported earlier by Denkert and colleagues (Denkert et al., 2018).

In our study, sTILs were not associated with the HER2 overexpression status. Then we also analyzed the association between sTILs and the clinicopathological features of breast cancer based on HER2 status. In HER2-negative breast cancer, TILs were significantly associated with higher histologic grade and higher Ki67 index, whereas no significant correlation was found in HER2-positive breast cancer.

Previous studies have documented high TILs levels in aggressive breast cancer subtypes and showed a possible relationship between high sTILs, Ki-67, ER, and PR immunohistochemical expression (Denkert et al., 2018). Similarly, in the present study, we observed a correlation between sTILs distribution, proliferative index (Ki-67), and hormone receptors. In detail, intermediate and high-levels of sTILs were significantly related to a high Ki-67 index.

sTILs played an important role, associated with unfavorable factors in breast cancer. Our findings support the use of stromal sTILs to identify a more aggressive phenotype of tumors. Further studies are needed to identify the effect between TILs and chemotherapy response.

Author Contribution Statement

Data curation: TTD, DTNP. Formal analysis: TTD, DTNP. Methodology: TTD, DTNP, HNTD, TTL. Supervision: TTD, TAT, HNTD, TTL. Writing—original draft: TTD, DTNP. Writing—review and editing: TTD, DTNP, TAT. Approval of final manuscript: all authors

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Approval

This paper is a part of the master's thesis at the University of Medicine and Pharmacy at Ho Chi Minh City.

Ethical Declaration

The study was approved by the Institutional Review Board of Ethics in Biomedical Research, University of Medicine and Pharmacy at Ho Chi Minh City (IRB No. 1008/UMP-BOARD; date: December 1, 2022) and performed by the principles of the Declaration of Helsinki.

Conflict of Interest

No potential competing interest was reported by the authors.

References

Anderson KN, Schwab RB, Martinez ME (2014). Reproductive risk factors and breast cancer subtypes: a review of the literature. *Breast Cancer Res Treat*, **144**, 1-10.

Anichini A, Fossati G, Parmiani G (1987). Clonal analysis of the cytolytic T-cell response to human tumors. *Immunol Today*, **8**, 385-9.

Baghban R, Roshangar L, Jahanban-Esfahlan R, et al (2020). Tumor microenvironment complexity and therapeutic implications at a glance. *Cell Commun Signal*, **18**, 59.

Bai Z, Zhou Y, Ye Z, et al (2021). Tumor-Infiltrating Lymphocytes in Colorectal Cancer: The Fundamental Indication and Application on Immunotherapy. *Front Immunol*, **12**, 808964.

Basu A, Ramamoorthi G, Jia Y, et al (2019). Immunotherapy in breast cancer: Current status and future directions. *Adv Cancer Res*, **143**, 295-349.

Bejarano L, Jordao MJC, Joyce JA (2021). Therapeutic Targeting of the Tumor Microenvironment. *Cancer Discov*, **11**, 933-59.

Board WCoTE 2019. WHO Classification of Breast Tumours: WHO Classification of Tumours, Volume 2, World Health Organization.

Castaneda CA, Mittendorf E, Casavilca S, et al (2016). Tumor infiltrating lymphocytes in triple negative breast cancer receiving neoadjuvant chemotherapy. *World J Clin Oncol*, **7**, 387-94.

De Wever O, Mareel M (2003). Role of tissue stroma in cancer cell invasion. *J Pathol*, **200**, 429-47.

Denkert C, Loibl S, Noske A, et al (2010). Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer. *J Clin Oncol*, **28**, 105-13.

Denkert C, von Minckwitz G, Brase JC, et al (2015). Tumor-infiltrating lymphocytes and response to neoadjuvant chemotherapy with or without carboplatin in human epidermal growth factor receptor 2-positive and triple-negative primary breast cancers. *J Clin Oncol*, **33**, 983-91.

Denkert C, von Minckwitz G, Darb-Esfahani S, et al (2018). Tumour-infiltrating lymphocytes and prognosis in different subtypes of breast cancer: a pooled analysis of 3771 patients treated with neoadjuvant therapy. *Lancet Oncol*, **19**, 40-50.

Dieci MV, Radosevic-Robin N, Fineberg S, et al (2018). Update on tumor-infiltrating lymphocytes (TILs) in breast cancer, including recommendations to assess TILs in residual disease after neoadjuvant therapy and in carcinoma in situ: A report of the International Immuno-Oncology Biomarker Working Group on Breast Cancer. *Semin Cancer Biol*, **52**, 16-25.

Fidler IJ (2003). The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. *Nat Rev Cancer*, **3**, 453-8.

Goldhirsch A, Winer EP, Coates AS, et al (2013). Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. *Ann Oncol*, **24**, 2206-23.

Hammond ME, Hayes DF, Dowsett M, et al (2010). American Society of Clinical Oncology/College Of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. *J Clin Oncol*, **28**, 2784-95.

Howlader N, Altekruse SF, Li CI, et al (2014). US incidence of breast cancer subtypes defined by joint hormone receptor and HER2 status. *J Natl Cancer Inst*, **106**.

Hudry D, Le Guellec S, Meignan S, et al (2022). Tumor-Infiltrating Lymphocytes (TILs) in Epithelial Ovarian Cancer: Heterogeneity, Prognostic Impact, and Relationship with Immune Checkpoints. *Cancers (Basel)*, **14**.

Issa-Nummer Y, Darb-Esfahani S, Loibl S, et al (2013). Prospective validation of immunological infiltrate for prediction of response to neoadjuvant chemotherapy in HER2-negative breast cancer--a substudy of the neoadjuvant GeparQuinto trial. *PLoS One*, **8**, e79775.

Kono-Sato T, Miyai K, Yamagishi Y, et al (2022). Intraepithelial lymphocytes are indicators of better prognosis in surgically resected endometrioid-type endometrial carcinomas at early and advanced stages. *BMC Cancer*, **22**, 361.

Kumar S, Kumar N, Deo S, et al (2022). Patterns of Multimodality Management of Gastric Cancer-Single Institutional Experience of 372 Cases From a Tertiary Care Center in North India. *Front Oncol*, **12**, 877493.

Kurozumi S, Matsumoto H, Kurosumi M, et al (2019). Prognostic significance of tumour-infiltrating lymphocytes for oestrogen receptor-negative breast cancer without lymph node metastasis. *Oncol Lett*, **17**, 2647-56.

Lin CH, Chuang PY, Chiang CJ, et al (2014). Distinct clinicopathological features and prognosis of emerging young-female breast cancer in an East Asian country: a nationwide cancer registry-based study. *Oncologist*, **19**, 583-91.

Loi S, Michiels S, Salgado R, et al (2014). Tumor infiltrating lymphocytes are prognostic in triple negative breast cancer and predictive for trastuzumab benefit in early breast cancer: results from the FinHER trial. *Ann Oncol*, **25**, 1544-50.

Mott JD, Werb Z (2004). Regulation of matrix biology by matrix metalloproteinases. *Curr Opin Cell Biol*, **16**, 558-64.

Naukkarinen A, Syrjanen KJ (1990). Quantitative immunohistochemical analysis of mononuclear infiltrates in breast carcinomas--correlation with tumour differentiation. *J Pathol*, **160**, 217-22.

Nguyen CV, Nguyen QT, Vu HTN, et al (2021). Molecular classification predicts survival for breast cancer patients in Vietnam: a single institutional retrospective analysis. *Int J Clin Exp Pathol*, **14**, 322-37.

Nielsen TO, Leung SCY, Rimm DL, et al (2021). Assessment of Ki67 in Breast Cancer: Updated Recommendations From the International Ki67 in Breast Cancer Working Group. *J Natl Cancer Inst*, **113**, 808-19.

Osipov A, Murphy A, Zheng L (2019). From immune checkpoints to vaccines: The past, present and future of cancer immunotherapy. *Adv Cancer Res*, **143**, 63-144.

Peppercorn J, Perou CM, Carey LA (2008). Molecular subtypes in breast cancer evaluation and management: divide and conquer. *Cancer Invest*, **26**, 1-10.

Perou CM, Sorlie T, Eisen MB, et al (2000). Molecular portraits of human breast tumours. *Nature*, **406**, 747-52.

Purushotham MK, Venkatesh PM (2021). Association of Histopathological Parameters and Axillary Lymphnode Metastasis in Primary Breast Carcinoma. *Asian Pac J Cancer Care*, **6**, 379-82.

Rouzier R, Perou CM, Symmans WF, et al (2005). Breast cancer molecular subtypes respond differently to preoperative chemotherapy. *Clin Cancer Res*, **11**, 5678-85.

Salgado R, Denkert C, Demaria S, et al (2015). The evaluation

of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. *Ann Oncol*, **26**, 259-71.

San TH, Fujisawa M, Fushimi S, et al (2017). Molecular Subtypes of Breast Cancers from Myanmar Women: A Study of 91 Cases at Two Pathology Centers. *Asian Pac J Cancer Prev*, **18**, 1617-21.

Stanton SE, Adams S, Disis ML (2016). Variation in the Incidence and Magnitude of Tumor-Infiltrating Lymphocytes in Breast Cancer Subtypes: A Systematic Review. *JAMA Oncol*, **2**, 1354-60.

Sung H, Ferlay J, Siegel RL, et al (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin*, **71**, 209-49.

Tang P, Tse GM (2016). Immunohistochemical Surrogates for Molecular Classification of Breast Carcinoma: A 2015 Update. *Arch Pathol Lab Med*, **140**, 806-14.

Wang M, Zhao J, Zhang L, et al (2017). Role of tumor microenvironment in tumorigenesis. *J Cancer*, **8**, 761-73.

Wolff AC, Hammond MEH, Allison KH, et al (2018). Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. *J Clin Oncol*, **36**, 2105-22.

Yamashita H, Iwase H, Toyama T, et al (2011). Estrogen receptor-positive breast cancer in Japanese women: trends in incidence, characteristics, and prognosis. *Ann Oncol*, **22**, 1318-25.

Yanagawa M, Ikemot K, Kawauchi S, et al (2012). Luminal A and luminal B (HER2 negative) subtypes of breast cancer consist of a mixture of tumors with different genotype. *BMC Res Notes*, **5**, 376.

Zhu X, Ying J, Wang F, et al (2014). Estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 status in invasive breast cancer: a 3,198 cases study at National Cancer Center, China. *Breast Cancer Res Treat*, **147**, 551-5.